Regulatory Toxicology and Pharmacology 81 (2016) 242–249

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Prediction of carcinogenic potential of chemicals using repeated-dose (13-week) toxicity data

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ARTICLE INFO

Article history:
Received 17 May 2016
Received in revised form
30 August 2016
Accepted 5 September 2016
Available online 8 September 2016

Keywords:
Rat
Sub-chronic toxicity
Carcinogenicity
Non-genotoxic carcinogens
Preneoplastic lesions
Tumours
Predictivity
Risk assessment

ABSTRACT

Sub-chronic toxicity studies of 163 non-genotoxic chemicals were evaluated in order to predict the tumour outcome of 24-month rat carcinogenicity studies obtained from the EFSA and ToxRef databases. Hundred eleven of the 148 chemicals that did not induce putative preneoplastic lesions in the sub-chronic study also did not induce tumours in the carcinogenicity study (True Negatives). Cellular hypertrophy appeared to be an unreliable predictor of carcinogenicity. The negative predictivity, the measure of the compounds evaluated that did not show any putative preneoplastic lesion in de sub-chronic studies and were negative in the carcinogenicity studies, was 75%, whereas the sensitivity, a measure of the sub-chronic study to predict a positive carcinogenicity outcome was only 5%. The specificity, the accuracy of the sub-chronic study to correctly identify non-carcinogens was 90%. When the chemicals which induced tumours generally considered not relevant for humans (33 out of 37 False Negatives) are classified as True Negatives, the negative predictivity amounts to 97%. Overall, the results of this retrospective study support the concept that chemicals showing no histopathological risk factors for neoplasia in a sub-chronic study in rats may be considered non-carcinogenic and do not require further testing in a carcinogenicity study.

1. Introduction

Cancer is one of the leading causes of death in industrialized countries. In those countries the cancer rate has risen from 1 in 10 in 1930 to 1 in 3 today. According to the American Cancer Society, almost 45% of men and 38% of women will be diagnosed with cancer at some point in their lives (American Cancer Society, 2014). Cancer is responsible for 7.6 million deaths worldwide per year, with 3 million new cancer cases per year in Europe alone (WHO, 2011).

Whilst there is no single cause of cancer, evidence is emerging that exposures to chemical substances in our everyday life may be contributing to the increasing cancer burden. Past occupational exposure to known or probable carcinogens is estimated to account for 5.3% (8023) of cancer deaths and 4% of cancer registration occurring each year in Great Britain (HSE, 2014).

Industry currently uses thousands of substances that have not yet been tested for their effect on human health. The EU REACH (Registration, Evaluation, Authorization and restriction of CHemicals) (EC, 2007) aims to evaluate any substance produced or imported in significant quantities unless sufficient safety information already exists. When applying the traditional toxicity tests this will cost more than 200 years to be completed. In the worst-case scenario, 2.9 million animals would be needed for testing all these chemicals (Van der Jagt et al., 2004). This is ethically and economically not defensible. Therefore, alternative methods for toxicity testing are warranted.

Much of what we know about chemicals and cancer comes from studies with animals, long-term follow-up of workers exposed to chemicals at their workplace, and epidemiological studies in

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http://dx.doi.org/10.1016/j.yrtph.2016.09.003

communities where residents are exposed to hazardous agents. Although a number of factors have been related to the induction of cancer, it is not possible to predict with complete certainty from animal studies alone which chemicals under which exposure circumstances will be carcinogenic in humans. The current 2-year rodent carcinogenicity study (OECD, 2009) has been the regulatory standard in safety assessments of environmental chemicals, and is presumed to forecast potential long-term human cancer risks of exposure to industrial chemicals. Under REACH, relevant factors that may trigger a two-year carcinogenicity bioassay in this respect include: i) genotoxicity (germ cell mutagen category 2); ii) evidence of treatment-related hyperplasia and/or preneoplastic lesions in the sub-chronic study; iii) previous demonstration of carcinogenic potential in the chemical class or product that is considered relevant to humans; iv) widespread use or evidence of frequent long-term human exposure.

A positive test result in an *in vivo* genotoxicity test is generally considered indicative of the carcinogenic potential of a chemical. For pharmaceuticals, agrochemicals and most consumer products, a positive result in an assay for DNA reactivity will usually preclude further development (ICH, 1995; Snyder and Green, 2001). Under REACH (EC, 2007), the requirement to perform a carcinogenicity study is conditional, since the default presumption is that if a substance is classified as germ cell mutagen category 1A or 1B, a genotoxic mechanism for carcinogenicity is likely.

There is, however, considerable scientific doubt about the reliability of the carcinogenicity bioassay, since too many false positive outcomes have been observed (Van Oosterhout et al., 1997; Cohen, 2004; Boobis et al., 2009) concerns raised about the predictive value of *in vivo* studies in general (Jacobson-Kram et al., 2004; Anisimov et al., 2005; Billington et al., 2010; Osimitz et al., 2013) and the push for refinement, reduction, and replacement of animal studies, resulted in the recommendation to re-evaluate the 2-year rodent bioassay as the best approach to predict human disease (Cohen, 2004; Ward, 2007; Boobis et al., 2009; Friedrich and Olejniczak, 2011; Sistare et al., 2011; Benigni, 2012; Doktorova et al., 2012; Gori, 2013).

Reddy et al. (2010) and Sistare et al. (2011) concluded that pharmaceuticals that showed absence of histopathological risk factors, such as hyperplasia, hypertrophy, foci of cellular alteration, and cell proliferation, for neoplastic lesions in any tissue in rats in the 6- or 12-month study may be considered non-carcinogens and do not require testing in a two-year carcinogenicity study, provided that these pharmaceuticals lack genotoxic potential and fail to induce hormonal perturbations.

The sub-chronic (13-week) rat study (OECD, 1998), which includes endpoints as clinical chemistry and histopathology of a wide range of organs and tissues, is currently required by regulatory bodies worldwide as the first tier in safety testing of foods and chemicals. In the present paper, we further investigated the hypothesis of Reddy et al. (2010) and Sistare et al. (2011) that chemicals showing no histopathological risk factors for carcinogenicity in an extended sub-chronic toxicity study may be considered non-carcinogenic (the so called “whole animal negative predictivity hypothesis”), using 202 chemicals for which adequate data from sub-chronic (13-week) and carcinogenicity (24-month) studies were present in the ToxRef EPA- and EFSA database.

2. Materials and methods

2.1. Data sources, and data inclusion and exclusion criteria

The rat sub-chronic toxicity and chronic carcinogenicity data used for this evaluation came from two different sources: the European Food Safety Agency (EFSA) database (www.efsa.europa.eu/publications) and the ToxRef EPA database (www.epagov/pesticides/science/comptox-glossary.html#toxrefdb). The data used are all publicly available and have been derived from studies performed according to OECD Test Guidelines.

This evaluation focused on sub-chronic (13-week) toxicity and carcinogenicity (24-month) studies conducted in rats. The criteria applied for inclusion of the studies were similar to those reported previously by Reddy et al. (2010) and Sistare et al. (2011). These are based on the study outcomes (putative preneoplastic lesions in sub-chronic studies, and tumours in carcinogenicity studies, resp.) and the dose levels used. Apart from a few exceptions, the short- en long-term studies were performed with the same strain of rats.

In short, studies were included in the evaluation when:

- the dose levels in the sub-chronic study showed any overlap with those of the carcinogenicity study;
- the sub-chronic study was negative (did not demonstrate putative preneoplastic lesions) at doses higher than those used in the carcinogenicity study;
- the sub-chronic study was positive (demonstrated putative preneoplastic histopathological changes) even if the doses were less than 75% of the doses used in the carcinogenicity study; or
- the sub-chronic study was negative and the top dose was less than 75% of the top-dose used in the carcinogenicity study, but lower doses in the carcinogenicity study matched those in de sub-chronic study and positive tumour findings occurred.

Substances were excluded on the basis of two criteria:

- the sub-chronic study was positive (demonstrated putative preneoplastic lesions) only at a dose that was over 25% higher than the highest dose used in the carcinogenicity study, and
- the highest dose in the sub-chronic study was negative (demonstrated no putative preneoplastic lesions), but this dose was less than 25% of the lowest dose in the carcinogenicity study.

2.2. Toxicological evaluation of substances

All substances present in the database were evaluated for the following parameters: body weight, organ weights, presence of putative preneoplastic histopathological changes in the sub-chronic study, treatment-related increased tumour incidences in the 24-month carcinogenicity studies, and genotoxicity. We have not included the mode or mechanism of action (e.g. hormonal perturbation) of the chemicals evaluated.

2.2.1. Histopathological changes

Substances were evaluated for the following histopathological changes in sub-chronic (13-week) toxicity studies:

- cellular hyperplasia,
- presence of altered hyperplastic foci of cellular alteration (atypical) cell foci (basophilic; acidophilic foci), and
- cellular proliferation.
- cellular hypertrophy.

In case of induction of cellular hypertrophy, this was recorded but not included as a putative preneoplastic lesion since an International ESTP Expert Workshop recently concluded that cellular hypertrophy without histopathological or clinical alterations are generally considered as an adaptive and non-adverse reaction and not as a step toward carcinogenicity (Hall et al., 2012).

Substances were scored negative when any of the